Food and Chemical Toxicology 48 (2010) 1152–1159

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits

Mokhtar I. Yousef *

Department of Home Economic, Faculty of Specific Education, Alexandria University, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 23 November 2009
Accepted 1 February 2010

Keywords:
Reproductive toxicity
Lambda-cyhalothrin
Vitamin E
Rabbits
Testosterone
Free radicals
Enzymes

ABSTRACT

The objective of the current study was to analyze the reproductive toxicity caused by lambda-cyhalothrin (LCT) in male rabbits, and to evaluate the possible protective effect of vitamin E (Vit. E) as antioxidant. Animals were orally administered their respective doses of LCT every other day and given drinking water supplemented with vitamin E for 16 weeks. Results showed that semen quality was deteriorated following treatment with LCT. Also, testosterone levels, body weight (BW), feed intake (FI), and relative testes (RTW) and epididymis (REW) weights were significantly decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly increased in seminal plasma of rabbits treated with LCT compared with control. While, activities of glutathione *S*-transferase (GST), transaminases and acid phosphatase (AcP) were significantly decreased. Vitamin E alone significantly increased testosterone levels, BW, FI, RTW, REW, semen characteristics and seminal plasma enzymes, and decreased the levels of TBARS. Also, the present study showed that vitamin E might be effective against LCT-induced reproductive toxicity. It was suggested that LCT exerted a significant adverse effect on reproductive performance of male rabbits. Furthermore, vitamin E antagonized the toxic effects of LCT and improved semen quality of male rabbit.

1. Introduction

Several currently used pesticides, especially those having endocrine disruptive properties, are known to adversely impair reproductive competence of males under laboratory, field, clinical or occupational settings (Figa-Talamanca et al., 2001). Some of these agents are among the most commonly used pesticides/insecticides in developing countries including Egypt. In many countries lambda-cyhalothrin has been successfully used for control of infectious disease vectors, such as mosquitoes, triotomine bugs and other arthropods (Awumbila and Bokuma, 1994). Lambda-cyhalothrin is a potent, synthetic, type II pyrethroid. It is a stomach, contact and a residual insecticide, which acts as a neuropoison interfering in the ionic conductance of nerve membranes by prolonging the sodium current (Clark, 1997). In addition, pyrethroids increase the spontaneous release of neurotransmitters such as GABA, dopamine or noradrenaline (Clark, 1997), and may also acts as hormone disruptor (Garey et al., 1999). Collectively, the facts suggest that lambda-cyhalothrin (Ratnasooriya et al., 2002) may disrupt male reproductive function, but this has not been experimentally documented.

Synthetic pyrethroids (sumithrin, fenvalerate, d-trans allethrin, permethrin, cypermethrin and fenvalerate) have ability to disrupt estrogen signaling and caused alterations in reproduction (Yousef et al., 2003a). Also, exposure to lambda-cyhalothrin caused sexual dysfunction in male rats (Ratnasooriya et al., 2002). During pyrethroid metabolism, reactive oxygen species (ROS) were generated and caused oxidative stress in intoxicated animals (Kale et al., 1999; Yousef et al., 2003a, 2006). Antioxidants can protect against the damaging effect of oxygen species on sperm quality (Baker et al., 1996; Yousef et al., 2003a,b, 2004, 2005, 2006, 2007; Yousef, 2004; Yousef and Salama, 2009). Overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Akiyama, 1999).

During the past few years, estimation of free radical generation and antioxidants defense has become an important aspect of investigation in mammals. Our recent studies were carried out to evaluate the potential role of antioxidant, such as, vitamin C, vitamin E, β-carotene, isoflavones, folic acid, propolis, curcumin and grape seed proanthocyanidin extract (Yousef, 2004; Yousef et al.,

Abbreviations: LCT, lambda-cyhalothrin; Vit. E, vitamin E; BW, body weight; FI, feed intake; RTW, relative testes weight; REW, relative epididymis weight; TBARS, thiobarbituric acid-reactive substances; GST, glutathione *S*-transferase; AST, aspartate transaminase; ALT, alanine transaminase; AcP, acid phosphatase; ROS, reactive oxygen species.

* Address: Department of Home Economic, Faculty of Specific Education, Alexandria University, 14 Mohamed Amin Shohaib Street, Moustafa Kamel, P.O. Box Roushdi, Alexandria 21529, Egypt. Tel.: +20 3 4252362/3 5454313; fax: +20 3 5442776.
E-mail address: yousefmokhtar@yahoo.com

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.02.002

2003a,b, 2004, 2005, 2006, 2007, 2008, 2009; Yousef and Salama, 2009) for the protection of cells against oxidative damage due to pesticides, heavy metals and chemotherapeutic agent toxicities.

Numerous antioxidants have proven beneficial in treating male infertility, such as vitamin C, vitamin E, glutathione, and coenzyme Q10 (Sinclair, 2000; Yousef et al., 2003a). Antioxidants can protect against the damaging effect of leukocyte-derived reactive oxygen species on sperm movement and may be of clinical value in assisted conception procedures (Baker et al., 1996). The production of reactive oxygen species (ROS) is a normal physiological event in various organs including the testis. Overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Akiyama, 1999).

Vitamin E is believed to be the primary components of the antioxidant system of the spermatozoa (Surai et al., 1998), and is one of the major membrane protectants against ROS and lipid peroxidation (Akiyama, 1999). Supplemental vitamin E has been shown to increase total sperm output and sperm concentration (Brzezinska-Slebodzinska et al., 1995) in boars. Also, vitamin E is a naturally occurring antioxidant nutrient that plays important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant function of vitamin E could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells (Chew, 1995). Because of the health problems induced by many environmental pollutants, much effort has been expended in evaluating the relative antioxidant potency of vitamin E. Therefore, the aim of this study was to assess (1) the potential impacts of lambda-cyhalothrin on body, testes and epididymis weights, plasma testosterone concentration and semen quality of male rabbits; (2) the role of vitamin E in alleviating the negative effects of lambda-cyhalothrin on reproductive characteristics.

2. Materials and methods

2.1. Chemicals

Lambda-cyhalothrin ($C_{23}H_{19}Cl–F_3NO_3$) was purchased from Kima Company, Egypt and vitamin E (Dietvit® E, 53% -tocopherol acetate), was manufactured by Codislait Sarl, 22120 Yffiniac, Neolait SA, France.

2.2. Animals and experimental design

Male New Zealand White rabbits (age of 7 months and initial weight of 2788 ± 32 gm) were used. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were individually housed in stainless steel cages. Feed and water were provided ad libitum. Rabbits were fed pellets consisted of 30% berseem (Trifolium alexandrinum) hay, 25% yellow corn, 26.2% wheat bran, 14% whole soybean meal, 3% molasses, 1% $CaCl_2$, 0.4% NaCl, 0.3% mixture of minerals and vitamins (0.01 gm/kg diet of vitamin E), and 0.1% methionine. The vitamin and mineral premix per kg contained the following vitamins: A-4,000,000 IU, D3–5000,000 IU, E-16,7 g, K-0.67 g, B1–0.67 g, B2–2 g, B6–0.67 g, B12–0.004 g, B5–16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g; and minerals: Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.).The chemical analysis of the pellets (AOAC, 1990) showed that they contained 17.5% crude protein, 14.0% crude fiber, 2.7% crude fat and 2200 K cal. digestable energy/kg diet.

Twenty-four mature male rabbits were randomly divided into four equal groups of six rabbits each. Group 1 served as control. However, group 2 was given drinking water supplemented with vitamin E (2 mg/kg body weight). Group 3 was orally given lambda-cyhalothrin (LCT) (20 mg/kg body weight) every other day. Group 4 was given the combination of LCT and vitamin E. The experiment was continued for 16-week. The proper doses of lambda-cyhalothrin for each animal were placed into a syringe that was inserted orally with the help of plastic tube directly into the oropharyngeal region. The doses of the lambda-cyhalothrin and vitamin E were calculated according to the animal's body weight on the week before dosing. The tested doses for lambda-cyhalothrin and vitamin E were given every other day for 16-weeks.

2.3. Semen collection and characteristics

Daily feed intake and body weight were recorded weekly. Semen collection occurred weekly over the 16 weeks of the study, so 96 ejaculates obtained per experimental group. Ejaculates collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. A weak eosin solution was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH+Co., Brandstwiete 4, 2000 Hamburg 11, Germany) (Smith and Mayer, 1955). Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma carried out immediately after collection according to Mann (1948). Assessment of live and abnormal spermatozoa was performed using an eosin–nigrosine blue staining mixture (Blom, 1950). The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. Total number of motile sperm calculated by multiplying percentage of motile sperm and total sperm outputs. Reaction time for the buck is calculated as the time needs for mounting a doe until complete ejaculation; it measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) parameter was also calculated as (total sperm output × motility (%) × normal morphology (%)) (Correa and Zavos, 1996).

2.4. Blood collection and testosterone determination

Blood samples were collected from the ear vein of each buck every other week and placed immediately on ice in heparinized tubes. Plasma was collected from blood by centrifuged at 860g for 20 min and stored at −60 °C. Testosterone concentration in plasma was measured by simple solid phase enzyme immunoassay utilizing horseradish peroxidase as a tracer (Equipar, via G. Ferrari, Saronno, Italy). Intra and interassay coefficient of variations were 3.9% and 6.2%, respectively. All rabbits were euthanized at the end of the experimental period (16 week). Weight of testis and epididymis was recorded.

2.5. Seminal plasma collection and Biochemical parameters

Seminal plasma was obtained by centrifugation of semen samples at 860 Xg for 20 min at 4 °C, and was stored at −60 °C until analysis. The activities of aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) activities were determined with kits from SENTINEL CH. (via principle Eugenio 5–20155 MILAN, Italy). The method of Moss (1984) was used to assay the activity of acid phosphatase (AcP; EC 3.1.3.2). *p*-Nitrophenyl phosphate is hydrolyzed in acid pH medium by the action of acid phosphatase. Liberated *p*-nitrophenyl is spectrophtometrically quantified. Seminal plasma glutathione *S*-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. (1974), using para-nitrobenzyl-chloride as a substrate. Thiobarbituric acid-reactive substances (TBARS) were measured in seminal plasma at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M-1Cm-1 was used for calculation (Tappel and Zalkin, 1959).

2.6. Statistical analysis

Data were analyzed as a randomized design (Steel and Torrie, 1981) using the General Linear Model procedure of SAS (1986). Dunnett post hoc analysis was used to compare means of treatment groups against the control. P values < 0.05 were accepted as significant.

3. Results

The present study showed that body weight (BW), relative weights of testes and epididymis, feed intake and plasma testosterone concentration were significantly ($P < 0.05$) decreased in rabbits treated with lambda-cyhalothrin as compared to either control or vitamin E-treated groups (Table 1). Vitamin E alone caused an increase ($P < 0.05$) in BW, relative weight of testes and epididymis, feed intake and plasma testosterone concentration as compared to control animals. In addition, co-treatment with vitamin E alleviated the toxicity of lambda-cyhalothrin with respect to the various tested parameters (Table 1).

Treatment with lambda-cyhalothrin decreased ($P < 0.05$) semen ejaculate volume (EV), packed sperm volume (PSV), sperm concentration, total sperm output (TSO), sperm motility (%), total motile sperm per ejaculate (TMS), total functional sperm fraction (TFSF), normal sperm, initial fructose and libido (by decreasing

Table 1
The overall means (±SE) of body weight (BW; g), feed intake (FI; g/kg BW/day), relative testes weight (RTW; g/100 g BW), relative epididymis weight (REW; g/100 g BW) and testosterone (nm/ml) during treatment of male rabbits with vitamin E (Vit. E), lambda-cyhalothrin (LCT) and/or their combination.

Parameter	Groups			
	Control	Vitamin E	LCT	LCT + vitamin E
BW	2962 ± 26[b]	3229 ± 29[a]	2211 ± 36[c]	2843 ± 41[b]
FI	49.5 ± 2.04[b]	56.2 ± 1.87[a]	35.2 ± 1.99[d]	46.8 ± 1.72[c]
RTW	0.162 ± 0.012[b]	0.213 ± 0.013[a]	0.107 ± 0.009[c]	0.142 ± 0.010[b]
REW	0.073 ± 0.005[b]	0.102 ± 0.007[a]	0.041 ± 0.004[b]	0.064 ± 0.006[b]
Testosterone	7.51 ± 0.44[b]	10.92 ± 0.57[a]	4.06 ± 0.29[c]	6.62.8 ± 0.36[b]

[a–d] Within row overall mean with different superscript letter differ significantly ($P < 0.05$).

Table 2
Effect of vitamin E (Vit. E), lambda-cyhalothrin (LCT) and/or their combination on semen characteristics of male rabbits (means ± SE).

Parameters	Groups			
	Control	Vitamin E	LCT	LCT + vitamin E
Ejaculate Volume (ml)	0.70 ± 0.02[b]	0.86 ± 0.02 [a]	0.55 ± 0.02[c]	0.67 ± 0.01[b]
Reaction Time (s)	6.4 ± 0.31[c]	4.7 ± 0.28[d]	12.9 ± 0.37[a]	6.7 ± 0.29[b]
pH	7. 7 ± 0.03[b]	7.2 ± 0.03[a]	8.5 ± 0.03[c]	7.9 ± 0.02[b]
Sperm concentration ($\times 10^6$/ml)	286 ± 8.9[b]	322 ± 7.4[a]	210 ± 5.6[d]	256 ± 9.3[c]
Total sperm output ($\times 10^6$)	202 ± 8.9[b]	283 ± 9.0[a]	117 ± 6.7[d]	170 ± 8.3[c]
Sperm motility (%)	73 ± 0.75[b]	80 ± 0.77 [a]	63 ± 1.05[d]	71 ± 0.84[b]
Total motile sperm ($\times 10^6$)	147 ± 6.5[b]	230 ± 7.8[a]	75 ± 6.1[d]	121 ± 6.9[c]
Dead sperm (%)	25 ± 0.38 [b]	18 ± 0.46 [c]	36 ± 0.99 [a]	27 ± 0.64 [b]
Normal sperm (%)	77 ± 1.34[c]	88 ± 2.29[d]	63 ± 1.28[a]	72 ± 2.38[b]
Packed sperm volume (%)	19.7 ± 0.18[b]	22.3 ± 0.18[a]	16.5 ± 0.19[c]	19.1 ± 0.17[b]
Initial fructose (mg/dl)	213 ± 6.2[b]	253 ± 8.4[a]	173 ± 4.9[d]	198 ± 6.0[b]
Total functional sperm fraction ($\times 10^6$)	102 ± 6.2 [b]	161 ± 4.4 [a]	48 ± 4.9[d]	87 ± 6.0[c]

The mean value represents 96 values for each treatment. Within row, means with different superscript letters (a–d) differ significantly ($P < 0.05$).

Table 3
The overall means (±SE) of seminal plasma thiobarbituric acid-reactive substances (TBARS), glutathione *S*-transferase, (GST), aspartate transaminase (AST), alanine transaminase (ALT) and acid phosphatase (AcP) during treatment of male rabbits with vitamin E (Vit. E), lambda-cyhalothrin (LCT) and/or their combination.

Parameter	Groups			
	Control	Vitamin E	LCT	LCT + vitamin E
TBARS*	0.91 ± 0.013[b]	0.53 ± 0.019[d]	1.81 ± 0.019[a]	1.13 ± 0.010[b]
GST**	1.03 ± 0.041[b]	1.58 ± 0.061[a]	0.56 ± 0.042[c]	1.01 ± 0.052[b]
AST (U/L)	36.2 ± 0.16[b]	45.7 ± 0.28[a]	18.2 ± 0.3[c]	35.1 ± 0.19[b]
ALT (U/L)	19.2 ± 0.14[b]	25.5 ± 0.25[a]	10.2 ± 0.29[c]	18.8 ± 0.13[b]
AcP (IU)	30.2 ± 0.51[b]	41.1 ± 0.78[a]	15.7 ± 0.94[b]	28.9 ± 0.69[b]

The mean value represents 96 values for each treatment. Within row, means with different superscript letters (a–d) differ significantly ($P < 0.05$).
* TBARS is expressed as nmol/ml.
** GST specific activity: mol/h.

the reaction time) as compared to control and vitamin E-treated rabbits groups (Table 2 and Figs. 1–4). In contrast, dead sperm and initial pH increased with lambda-cyhalothrin exposure compared to controls. On the other hand, vitamin E improved semen quality. Vitamin E also counteracted or suppressed the adverse effects of lambda-cyhalothrin on semen characteristics (Table 2 and Figs. 1–4).

Our results showed also that there was a significant ($P < 0.05$) elevation in the concentrations of thiobarbituric acid-reactive substances (TBARS) in seminal plasma of lambda-cyhalothrin treated animals compared to control and vitamin E-treated groups (Table 3). However, the activities of glutathione-*S*-transferase (GST), aspartate transaminase (AST), alanine transaminase (ALT) and acid phosphatase (AcP) were significantly decreased ($P < 0.05$) in seminal plasma of lambda-cyhalothrin group as compared to either control or vitamin E-treated groups (Table 3). Treatment with vitamin E alone decreased ($P < 0.05$) the levels of seminal plasma TBARS and increased the activities of GST, AST, ALT and AcP (Table 3). In addition, vitamin E supplementation to lambda-cyhalothrin-treated rabbits significantly ($P < 0.05$) reduced the level of TBARS, and maintained the enzyme activities to the normal values compared to the control group.

4. Discussion

4.1. Lambda-cyhalothrin treatment

Treatment with lambda-cyhalothrin reduced testosterone levels, feed intake, body weight (BW) and relative testes (RTW) and epididymis (REW) weights (Table 1). Also, Ratnasooriya et al. (2002) reported that lambda-cyhalothrin showed signs of toxicity (reduction in food intake, diarrohea, suppression in body weight gain, ataxia, lethargy, sedation, haemotoxicity). Moreover, previous studies also showed a decrease in these parameters in rabbits treated with cypermethrin (Yousef et al., 2003a). Yousef et al. (1995) reported that the reduction of body weight of rabbits treated with carbofuran and glyphosate may be due to direct cytotoxic effect of the pesticides on somatic cells, and/or indirectly through the central nervous system which control feed and water intake and regulates the endocrine function. Also, the failure of different species exposed to environmental toxicant to gain body weight may be due to the decrease in feed intake, malabsorption of nutrients from the gastrointestinal tract and impaired feed conversion efficiency (Ball and Chhabra, 1981). The decline in the BW of treated rabbits with lambda-cyhalothrin appeared as a result of lesser intake of feed (Table 1).

Table 1 shows that relative weight of testes and epididymis, and testosterone levels were reduced by lambda-cyhalothrin treatment and these results are in agreement with the findings by Elbetieha

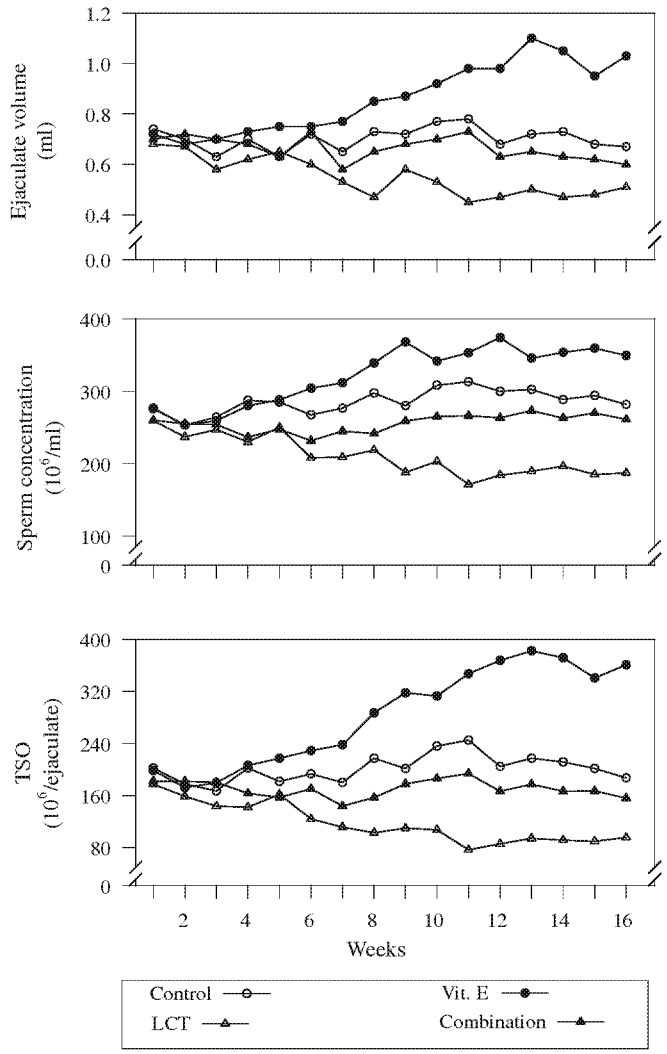

Fig. 1. Changes in ejaculate volume, sperm concentration and total sperm output (TSO) during treatment of male rabbits with vitamin E (Vit. E), lambda-cyhalothrin (LCT) or their combination.

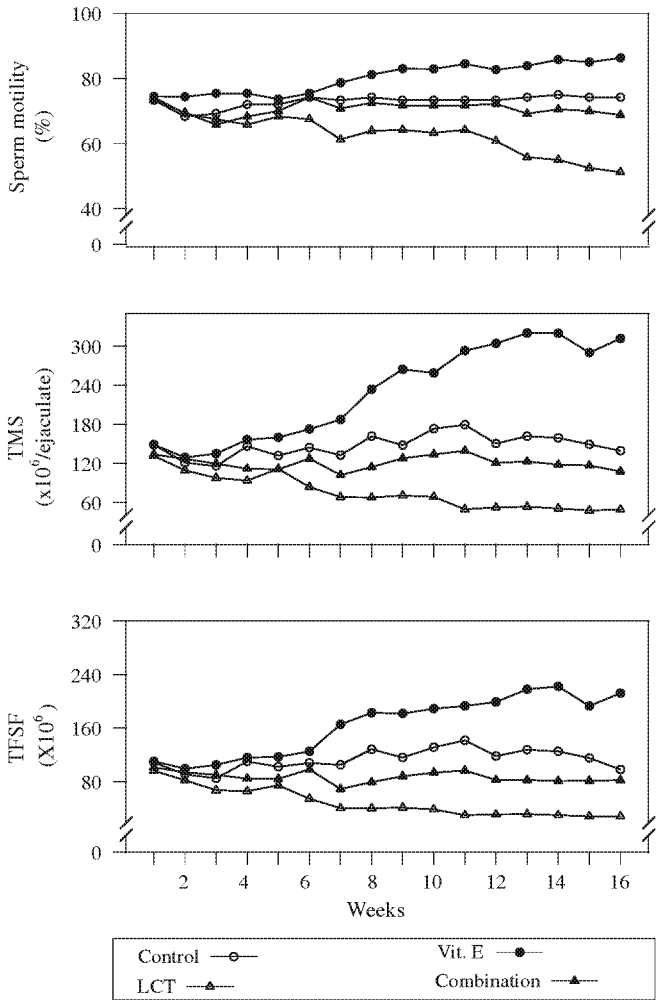

Fig. 2. Changes in motility, total motil sperm (TMS) and total functional sperm fraction (TFSF) during treatment of male rabbits with vitamin E (Vit. E), lambda-cyhalothrin (LCT) or their combination.

et al. (2001) in rats and Yousef et al. (2003a) in rabbits. These changes could be partly attributed to decreased feed consumption (Table 1). Elbetieha et al. (2001) found that serum levels of testosterone, follicle-stimulating hormone and luteinzing hormone were significantly reduced in male rats exposed to cypermethrin. Also, Moore et al. (1985) reported that 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) caused decreases in plasma testosterone and dihydraxy testosterone (DHT) and also decreases seminal vesicle, ventral prostate, caput epididymis, and testes weights, and adversely affects spermatogenesis. These effects could be attributed to hypophagia or weight loss because nutritional status is known to affect the male reproductive system, and TCDD may also affect hormones which regulate the male reproductive system and/or hormones which are produced by it. Goldman et al. (1990) indicated that chlordimeform (CDF) is able to affect markedly endocrine regulatory signals within the male rat reproductive system. Concentrations of serum gonadotropins (LH and FSH) and testosterone were all significantly declined and these declines are consistent with an effect on the transmitter control of hypothalamic gonadotropin-relasing hormone (GnRH) release. These effects on testosterone were attributed to the fall in serum LH, since LH serves as a normal stimulus for the secretion of this steroid from the testicular Lydig cells. It is also possible that there is a direct influence of CDF on testicular tissue, since adrenergic receptors are present in rat and mouse Leydig cells. In our study, the lambda-cyhalothrin-induced reduction in testosterone secretion can also be attributed to the reduction in testes weight (Table 1).

Semen quality (Table 2 and Figs. 1–4) deteriorated following treatment with lambda-cyhalothrin and these results are in agreement with the previous studies (Ratnasooriya et al., 2002; Yousef et al., 2003a). Exposure to lambda-cyhalothrin caused sexual dysfunction in male rats (Ratnasooriya et al., 2002). The decline in ejaculate volume, sperm concentration, total sperm output, and packed sperm volume (PSV), and increased reaction time can be partly attributed to the lambda-cyhalothrin-induced reduction in testosterone levels (Table 1). The effects of pesticides on spermatogenesis may be mediated through their effects on hormonal balance. Previous studies showed reduced semen quality in men occupationally exposed to various pesticides (Padungtod et al., 1998) and in animals (Yousef et al., 1995). Additionally, Elbetieha et al. (2001) found that treatment with cypermethrin caused reduction in the fertility of male rats. Also, the epididymal and testicular sperm counts as well as daily sperm production were significantly decreased and the number of implantation sites was significantly reduced in females mated with males that had ingested cypermethrin. The decrease in sperm packed volume of

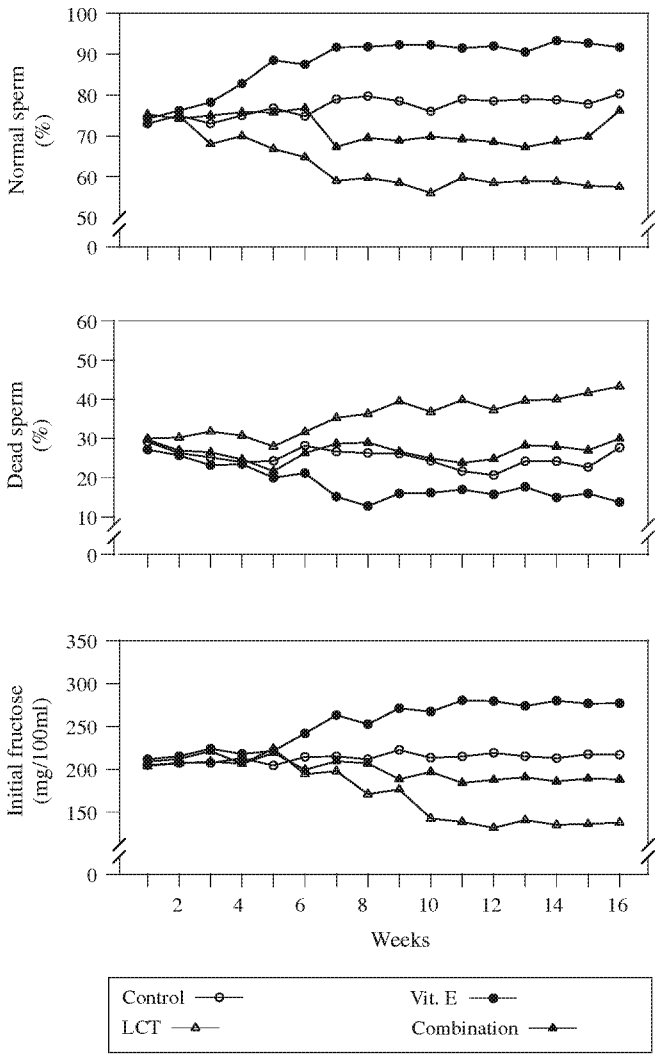

Fig. 3. Changes in normal sperm, dead sperm and initial fructose during treatment of male rabbits with E (Vit. E), lambda-cyhalothrin (LCT) or their combination.

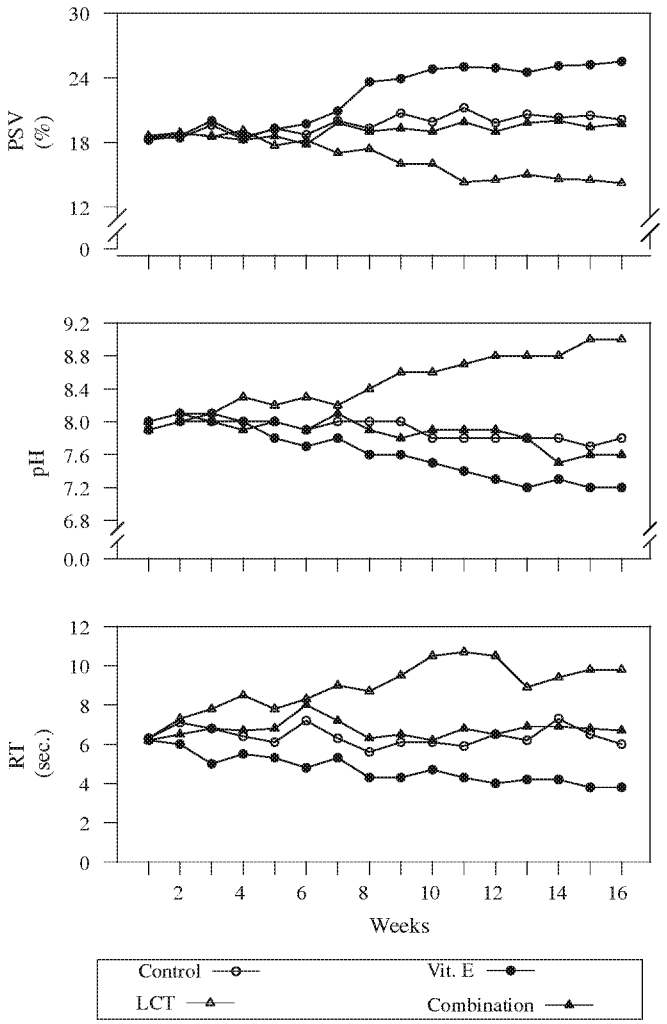

Fig. 4. Changes in packed sperm volume (PSV), initial hydrogen ion (pH) and reaction time (RT) during treatment of male rabbits with vitamin E (Vit. E), lambda-cyhalothrin (LCT) or their combination.

treated rabbits with lambda-cyhalothrin was coincided with the decrease in sperm concentration and total sperm output (Table 2).

Lambda-cyhalothrin increased pH and RT (Table 2 and Fig. 4) and these results agree with the finding by Yousef et al. (1995, 2003a). Harraway et al. (2000) stated that the normal pH of semen ranges from 7.2 to 8.0. Kuo et al. (1997) reported that semen pH were significant factors that explained the semen count and semen motility results. Graves (1978) reported that good-quality semen is usually on the acid side of neutrality than semen with lower sperm concentration, and semen containing many dead spermatozoa may evolve ammonia, which will increase the pH value. Accordingly, in the present investigation, the significant increase in pH value in lambda-cyhalothrin-treated group may be due to the increase of dead and abnormal sperm, and decrease sperm concentration (Table 2 and Figs. 1–4).

Effect of lambda-cyhalothrin on sperm morphology, viability and semen initial fructose concentration was observed in this study (Table 2 and Fig. 3). The present data showed that treatment with lambda-cyhalothrin caused significant ($P < 0.05$) increase in the percentage of dead sperm, while the percentage of abnormal sperm and semen initial fructose significantly ($P < 0.05$) decreased. These results agree with the finding by Yousef et al. (1995, 2003a) who reported that rabbits treated with pesticides showed significant increase in dead and abnormal sperm and reduction in initial fructose. Yousef et al. (2003a) indicated that cypermethrin had damaging effects on spermatogenesis and increase dead and abnormal sperm, and this disturbance was due to the decrease in the levels of testosterone. The fructose formation by the accessory glands is dependent upon the secretion of testosterone by the testes (Atterwill and Steele, 1987). Also, Hiro et al. (1963) stated that the decreased fructose content in rabbits semen is considered to be due to decreased androgen secretion by the testes. Accordingly, the observed reduction in initial fructose suggests a corresponding decrease in testosterone secretion by pesticide treatment (Table 1).

Results showed that treatment with lambda-cyhalothrin caused a significant ($P < 0.05$) decrease in sperm motility (Table 2 and Fig. 2). Motility is involved in defining the ability of the spermatozoa to ascend the reproductive tract to the site of fertilization, as well as the act of fertilization itself, particularly regarding the penetration of the vestments surrounding the oocyte, including the cumulus oophorus and zona pellucida. In view of the significance of sperm motility, it is not surprising that this criterion of sperm function has assumed a central role in the routine clinical diagnosis of male fertility (Aitken, 1990). Thus, high quality semen should contain a high percentage of vigorous and active sperms and should have higher glycolytic or fructolytic rates than do weak

immobile sperms. Fructose synthesis and secretion by the accessory glands is dependent upon the secretion of testosterone by the testes (Mann, 1964). Kim and Parthasarathy (1998) reported that generation of ROS and peroxidation of sperm membranes could bring negative effects on motility, midpiece abnormalities and sperm-oocyte fusion. Also, our results showed increased TBARS (Table 3) and decreased sperm motility (Table 2). Thus, the observed decrease in sperm motility could be attributed in part to the concomitant reduction in serum testosterone concentration (Table 1) and semen fructose (Fig. 3) following the lambda-cyhalothrin treatment. Yousef et al. (1996) suggested that pesticide's disruption of reproductive processes might be in part due to adverse effects on sperm cell function. In general the effect of lambda-cyhalothrin on sperm quality may be due to the decrease in plasma testosterone concentration and/or indirectly by reducing feed intake (Table 1).

Rats treated with lambda-cyhalothrin had markedly impaired libido as measured by suppression of pre-coital sexual behaviour (qualitatively), index of libido, and numbers of rats mounting, intromitting or ejaculating (Ratnasooriya et al., 2002). The rapid onset and equally rapid reversibility of these effects suggest that the anti-libido action of lambda-cyhalothrin was not owing to changes in the blood testosterone or prolactin levels. Both testosterone deficiency and hyperprolactaemia inhibit libido (Bartke et al., 1997). Lambda-cyhalothrin can release GABA and dopamine (Clark, 1997). GABA agonists, dopamine or dopamine agonists inhibits sexual behaviour in rats more or less in a similar fashion (Agmo et al., 1997). Thus lambda-cyhalothrin may induce anti-libido effects probably via brain GABAergic and/or dopaminergic systems (Agmo et al., 1997). In addition, lambda-cyhalothrin through its neurotoxicity could directly inhibit the sexual center in the hypothalamus and thereby suppress libido (Clark, 1997). Alternatively, lambda-cyhalothrin may induce anti-libido effects through cholinergic mechanisms, as pronounced cholinergic side effects were evident in the study and cholinergic agonist suppress libido (Retana-Marquez et al., 1993). Lambda-cyhalothrin treatment induced detrimental changes on health and behaviour, which are usually considered as manifestation of general toxicity. Thus, a strong possibility exists that lambda-cyhalothrin induced reduction of libido is secondary to its general toxicity.

The high generation of reactive oxygen species (ROS) as evidenced from significantly increased thiobarbituric acid-reactive substances (TBARS) levels and decreased antioxidant enzyme glutathione *S*-transferase (GST) in seminal plasma (Table 3) of lambda-cyhalothrin-treated rabbits is in agreement with our previous reports (Yousef et al., 2003a); hence, lambda-cyhalothrin-induced deterioration in semen quality is likely due to ROS-mediated membrane damage through impairment of membrane fluidity and permeability of the polyunsaturated membranes. Testicular mitochondria, microsomes, peroxisome and cytosol may generate ROS during normal metabolism that may be enhanced or accelerated in cells exposed to xenobiotics, pesticides and radiation; therefore, testicular cells are equipped with antioxidant systems. In comparison to other organs the testes contains very high levels of glutathione (GSH), which is believed to play an important role in the proliferation and differentiation of spermatogenic cells while simultaneously protecting these cells from ROS damage (Teaf et al., 1985). The decrease in GST activity corroborated the findings of Yousef et al. (2004, 2005, 2006, 2007) of decreased activities of testicular antioxidant enzymes in rabbits. Thus, the observed increase in free radicals could be attributed in part to the concomitant reduction of GST activity following lambda-cyhalothrin treatment. The transaminases (AST and ALT) and acid phosphatase (AcP) in semen play an important role in transamination and phosphorylation processes in sperm metabolism (Dhami et al., 1994). The present results revealed a significant ($P < 0.05$) decrease in the activities of seminal plasma AST, ALT and AcP of rabbits treated with lambda-cyhalothrin (Table 3). The decrease in the activities of these enzymes may be due to the decrease in the secretory activity of male accessory sex glands. Dhami et al. (1994) reported that the activities of AST, ALT, AcP, AlP and LDH were lower in static than in motile ejaculates of bulls. Also, our results revealed that the decrease in the activities of seminal plasma enzymes (Table 3) was coincided with the decrease of semen quality of treated rabbits with lambda-cyhalothrin (Table 3).

4.2. Vitamin E treatment

Treatment with vitamin E alone caused a significant ($P < 0.05$) increase in body weight and relative testes and epididymis weights (Table 1). Shetaewi (1998) found that supplementation of vitamin E to California and New Zealand White rabbits increased body weight gain and improved feed efficiency compared to the control group. Our previous studies showed that vitamin E supplementation stimulated weight gain in rabbits (Yousef et al., 2003b) which is in agreement with the present results. The beneficial effects of vitamin E noted in the present study can be attributed to the antioxidant effects of this vitamin; it is scavenger of oxygen-free radicals which are toxic byproducts of many metabolic processes (Meydani, 1995; Yousef et al., 2003b, 2006, 2007).

Vitamin E protects critical cellular structures against damage caused by oxygen-free radicals and reactive products of lipid peroxidation. It has been reported that lipid peroxidation was prevented by vitamin E (Meydani, 1995; Yousef et al., 2006). Vitamin E inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals, as a consequence of which it is converted into α-tocopheroxyl radical. This radical is thought to be either recycled to α-tocopherol by interacting with soluble antioxidants, such as ascorbic acid, or irreversibly oxidized to α-tocopherylquinone. In fact, α-tocopherylquinone may act as a potent anticoagulant, and as an antioxidant through its reduction to hydroquinone (Arita et al., 1998). Also, Boldyrev et al. (1995) reported that the protective role of vitamin E against the toxicity of oxidants may be due to the quenching of hydroxyl radicals.

Treatment with vitamin E alone caused a significant ($P < 0.05$) increase in semen quality, and minimized the toxic effects of lambda-cyhalothrin (Table 2 and Figs. 1–4). Yousef et al. (2003b, 2006, 2007) reported that the formation of TBARS was significantly ($P < 0.05$) decreased by treatment with vitamin E alone. Similarly, Hsu et al. (1998) reported that vitamin E supplementation reduced ROS generation and protected spermatozoa from loss of motility. Geva et al. (1996) found that oral treatment with 200 mg vitamin E daily decreased ROS significantly and increased fertilization rate of fertile normospermic human male after one month of treatment. Also, Yousef et al. (2003b) reported that treatment with vitamin E decreased the formation of TBARS and improved semen quality of rabbits. In addition, *in vitro* study using rabbit sperm by Yousef et al. (2007) showed that vitamin E decreased TBARS and increased antioxidant enzymes (superoxide dismutase and catalase). They also reported that supplementation with vitamin E was more effective in improving sperm characteristics and in reducing the production of reactive oxygen species than Vitamin C. Brzezinska-Slebodzinska et al. (1995) reported that *in vivo* experiments, seven weeks of oral vitamin E (1000 IU/d/animal) administration in boar caused a significant fall in the level of seminal plasma TBARS from 2.2 to 1.2 nmol/ml and significantly increased the number of spermatozoa. Therefore, the improving effect of vitamin E on semen characteristics may be due to the reduction in lipid peroxidation potential. The ameliorating effect of vitamin E (Table 2) against the toxicity of lambda-cyhalothrin on semen quality may be due to their role as antioxidant through quenching $^1O_2$ or free radical and reacting with peroxyl radicals (El-Missiry and Shalaby, 2000).

5. Conclusion

From the present results, it can be concluded that concurrent administration of vitamin E to lambda-cyhalothrin-treated animals ameliorated the induced sperm quality damage, significantly improved the sperm parameters and reduced the induction of seminal plasma free radicals. This is consistent with a vital role of vitamin E in antioxidant systems that protect against lambda-cyhalothrin damage, possibly by preventing oxidative damage to sperm. The present study suggest therapeutic effects of vitamin E to minimize the reproductive toxicity of lambda-cyhalothrin exposure.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Agmo, A., Paredes, R.G., Sierra, L., Garces, I., 1997. The inhibitory effects on sexual behaviour and ambulatory activity of the mixed GABAA/GABAB agonist progabide are differentially blocked by GABA receptor agonists. Psychophormacology 129, 27–34.

Aitken, R.J., 1990. Development of in vitro tests of human sperm function: a diagnostic tool and model system for toxicological analyses. Toxicol. *In Vitro* 4, 560–569.

Akiyama, M., 1999. *In vivo* scavenging effect of ethylcysteine on reactive oxygen species in human semen. Nippon Hinyokika Gakkai Zasshi 90, 421–428.

AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Agricultural Chemists, 13th ed. Benjamin, Franklin Station, Washington, DC.

Arita, M., Sato, Y., Arai, H., Inoue, K., 1998. Binding of atocopherylquinone, an oxidized form of a-tocopherol, to glutathione *S*-transferase in the liver cytosol. FEBS Lett. 436, 424–426.

Atterwill, C.K., Steele, C.E., 1987. In Vitro Methods in Toxicology. Cambridge University Press 411, 424.

Awumbila, B., Bokuma, E., 1994. Survey of pesticides used in the control of ectoparasites on farm animals in Ghana. Trop. Anim. Health Prod. 26, 7–12.

Baker, H.W., Brindle, J., Irvine, D.S., Aitken, R.J., 1996. Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes. Fertil. Steril. 65, 411–419.

Ball, L.M., Chhabra, R.S., 1981. Intestinal absorption of nutrients in rats treated with 2, 3, 7, 8-tetrachlorodi benz-*p*-dioxin (TCDD). J. Toxicol. Environ. Health 8, 629–636.

Bartke, A., Smith, M.S., Michael, S.D., Person, F.G., Darkterio, S., 1997. Effects of experimentally induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. J. Endrocrinol. 49, 311–316.

Blom, E., 1950. A one-minute live-dead sperm stain by means of eosin–nigrosin. J. Fertil. Steril. 1, 176–177.

Boldyrev, A.A., Bulygina, E.R., Volynskaya, E.A., Kurella, E.G., Tyulina, O.V., 1995. Effect of hydrogen peroxide and hypochlorite on the activity of Na, K-ATPase from brain. Biochemistry 60, 1293–1298.

Brzezinska-Slebodzinska, E., Slebodzinski, A.B., Pietras, B., Wieczorek, G., 1995. Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biol. Trace Elem. Res. 47, 69–74.

Chew, B.P., 1995. Antioxidant vitamins affect food animal immunity and health. J. Nutr. 125, 1804–1808.

Clark, J.M., 1997. Insecticides as tools in probing vital receptors and enzymes in excitable membranes. Pesticide Biochem. Physiol. 57, 235–254.

Correa, J.R., Zavos, P.M., 1996. Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. Theriogenology 46, 1225–12232.

Dhami, A.J., Sahni, K.L., Mohan, G., Tripathi, R.P., 1994. Comparative evaluation of initially static and motile semen ejaculate from Frieesian and Murrah buffalo bulls for physico-morphological, biochemical, enzymatic and mineral constituents of seminal plasma. Indian J. Anim. Sci. 64, 926–932.

Elbetieha, A., Da'as, S.I., Khamas, W., Darmani, H., 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch. Environ. Contam. Toxicol. 41, 522–528.

El-Missiry, M.A., Shalaby, F., 2000. Role of beta-carotene in ameliorating the cadmium-induced oxidative stress in rat brain and testis. J. Biochem. Mol. Toxicol. 14, 238–243.

Figa-Talamanca, I., Traina, M.E., Urbani, E., 2001. Occupational exposures to metals, solvents and pesticides: recent evidence on male reproductive effects and biological markers. Occup. Med. (London) 51, 174–188.

Garey, J., Wolff, M.S., Beatriz, G.T., 1999. Estrogenic potential of certain pyrethroid compounds in the MCF-7 human breast carcinoma cell line. Environ. Health Perspect. 107, 173–177.

Geva, E., Bartoov, B., Zabludovsky, N., Lessing, J.B., Lerner-Geva, L., Amit, A., 1996. The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. Fertil. Steril. 66, 430–434.

Goldman, J.M., Cooper, R.L., Laws, S.C., Rehnberg, G.L., Edwards, T.L., McElroy, W.K., Hein, J.F., 1990. Chlordimeform-iduced alterations in endocrine regulation within the male rat reproductive system. Toxicol. Appl. Pharmacol. 104, 25–35.

Graves, C.N., 1978. Semen and its components. In: Salisbury, G.W., Van Demark, N.L., Lodhe, J.R. (Eds.), Physiology of Reproduction and Artificial Insemination of Cattle, second ed. W.H. Freeman and Company, San Francisco, p. 247.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Harraway, C., Berger, N.G., Dubin, N.H., 2000. Semen pH in-patients with normal versus abnormal sperm characteristics. Am. J. Obstet. Gynecol. 182, 1045–1047.

Hiro, K., Tomitsuka, T., Massaki, J., 1963. Seasonal variations of fructose concentration in the seminal plasma of rams, goats, bul, boars and rabbits. Bull. Nat. Inst. Anim. Ind. (China) 7, 189 (A. B. A., 31: 2200).

Hsu, P.C., Liu, M.Y., Hsu, C.C., Chen, L.Y., Guo, Y.L., 1998. Effects of vitamin E and/or C on reactive oxygen species-related lead toxicity in the rat sperm. Toxicology 128, 169–179.

Kale, M., Rathore, N., John, S., Bhatnagar, D., 1999. Lipid peroxidative damage of pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxcicol. Lett. 105, 197–205.

Kim, J.G., Parthasarathy, S., 1998. Oxidation and the spermatozoa. Semen Reprod. Endocrinol. 16, 235–239.

Kuo, H.W., Wang, C.S., Lai, J.S., 1997. Semen quality in workers with long-term lead exposure: a preliminary study in Taiwan. Sci. Total Environ. 204, 289–292.

Mann, T., 1948. Fructose content and fructolysis in semen: Practical application in the evaluation of semen quality. J. Agric. Sci. (Camb.) 38, 323–331.

Mann, T., 1964. Fructose, polylos, and organic acids. In: The Biochemistry of Semen and the Male Reproductive Tract. John Wiley and Sons Inc., New York, US, pp. 237–264.

Meydani, M., 1995. Vitamin E. Lancet 345, 170–175.

Moore, R.W., Potter, C.L., Michael Theobaled, H., Robinson, J.A., Peterson, R.E., 1985. In utero and lactational exposure of male rats to 2, 3, 7, 8-tetrachlorodibenzeno-*p*-dioxin. Effects on androgenic status. Toxicol. Appl. Pharmacol. 114, 97–107.

Moss, D.W., 1984. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis, 3rd ed., vol. 4. Verlag-Chemie, pp. 92–106.

Padungtod, C., Lasley, B.L., Chritiani, D.C., Ryan, L.M., 1998. Reproductive hormone profile among pesticide factory workers. J. Occup. Environ. Med. 40, 1038–1047.

Ratnasooriya, W.D., Ratnayake, S.S.K., Jayatunga, Y.N.A., 2002. Effects of pyrethroid insecticide ICON (lambda-cyhalothrin) on reproductive competence of male rats. Asian J. Androl. 4, 35–41.

Retana-Marquez, S., Salazar, E.D., Velazquez-Moctezuma, J., 1993. Muscarinic and nicotinic influences on masculine sexual behaviour in rats: effects of oxotremorine, scopolamine and nicotine. Pharmacol. Biochem. Behav. 44, 913–917.

SAS, 1986. Statistical Analysis System. SAS User's Guide: Statistics, Version 5 Edition SAS Inst., Inc., Cary, NC, USA.

Shetaewi, M.M., 1998. Efficacy of dietary high levels of antioxidant Vitamins C and E for rabbits subjected to crowding stress. Egypt J. Rabbit Sci. 8, 95–112.

Sinclair, S., 2000. Male Infertility: Nutritional and Environmental Considerations. Green Valley Health, Hagerstown, MD 21742, USA.

Smith, J.T., Mayer, D.T., 1955. Evaluation of sperm concentration by the hemocytometer method. Fertil. Steril. 6, 271–275.

Steel, R.G.D., Torrie, J.H., 1981. Principle and Procedure of Statistics. A Biometrical Approach, second ed. Mc Gvaus-Hill Booh Company, New York, USA.

Surai, P., Kostjuk, I., Wishart, G., Macpherson, A., Speake, B., Noble, R., Ionov, I., Kutz, E., 1998. Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes, and liver. Biol. Trace Elem. Res. 64, 119–132.

Tappel, A.L., Zalkin, H., 1959. Inhibition of lipid peroxidation in mitochondria by vitamin E. Arch. Biochem. Biophys. 80, 333–336.

Teaf, C.M., Harbison, R.D., Bishop, B., 1985. Germ-cell mutagenesis and GSH depression in reproductive tissue of the F-344 rat induced byethyl methane sulfonate. Mutat. Res. 144, 93–98.

Yousef, M.I., 2004. Protective role of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. Toxicology 207, 81–89.

Yousef, M.I., Salama, A.F., 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol. 47, 1168–1175.

Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K., 1995. Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. J. Environ. Sci. Health B 30 (4), 513–534.

Yousef, M.I., Bertheussen, K., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Salem, M.H., 1996. A sensitive sperm-motility test for the assessment of cytotoxic effect of pesticides. J. Environ. Sci. Health B31, 99–115.

Yousef, M.I., Abdallah, G.A., Kamel, K.I., 2003a. Effect of ascorbic acid and vitamin E supplemintation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci. 76, 99–111.

Yousef, M.I., El-Demerdash, F.M., Al-Salhen, K.S., 2003b. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J. Environ. Sci. Health B38, 463–478.

Yousef, M.I., Ismail, A.M., Baghdadi, H.H., 2004. Effect of isoflavones on reproductive performance, testosterone levels, lipid peroxidation and seminal plasma biochemistry of male rabbits. J. Environ. Sci. Health B39, 819–833.

Yousef, M.I., El-Morsy, A.M.A., Hassan, M.E., 2005. Aluminium-induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: protective role of ascorbic acid. Toxicology 215, 97–107.

Yousef, M.I., Awad, T.T.I., Mohamed, E.H., 2006a. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology 227, 240–247.

Yousef, M.I., El-Demerdash, F.M., Kamil, K.I., Elaswad, F.A.M., 2006b. Ameliorating effect of folic acid on chromium(VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. Reprod. Toxicol. 21, 322–326.

Yousef, M.I., Kamil, K.I., El-Guendi, M.I., El-Demerdash, F.M., 2007. An *in vitro* study on reproductive toxicity of aluminium chloride on rabbit sperm: the protective role of some antioxidants. Toxicology 239, 213–223.

Yousef, M.I., El-Demerdash, F.M., Radwan, F.M.E., 2008. Sodium arsenite-induced biochemical perturbations in rats: ameliorating effect of curcumin. Food Chem. Toxicol. 46, 3506–3511.

Yousef, M.I., Saad, A.A., El-Shennawy, L.K., 2009. Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem. Toxicol. 47, 1176–1183.